Original Article

Cytogenetic and Genome Research

Cytogenet Genome Res 2011;134:144–150
DOI: 10.1159/000326764

Accepted: January 24, 2011
by M. Schmid
Published online: May 5, 2011

Genetic Compensation Abilities of *Aegilops speltoides* Chromosomes for Homoeologous B-Genome Chromosomes of Polyploid Wheat in Disomic S(B) Chromosome Substitution Lines

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Key Words
Aegilops speltoides · Karyotype evolution · *Triticum aestivum*

Abstract
The S genome of *Aegilops speltoides* is closely related to the B and G genomes of polyploid wheats. However, little work has been reported on the genetic relationships between the S-genome and B-genome chromosomes of polyploid wheat. Here, we report the isolation of a set of disomic substitutions (DS) of S-genome chromosomes for the B-genome chromosomes and their effects on gametophytic and sporophytic development. *Ae. speltoides* chromosomes were identified by their distinct C-banding and fluorescence in situ hybridization patterns with the *Ae. speltoides*-derived clone pGc1R-1. Although no large structural differences between S-genome and B-genome chromosomes exist, significant differences in gametophytic compensation were observed for chromosomes 1S, 3S, 5S and 6S. Similarly, chromosomes 1S, 2S, 4S, 5S and 6S affected certain aspects of sporophytic development in relation to spike morphology, fertility and meiotic pairing. The DS5S(5B) had disturbed meiosis with univalents/multivalents and suffered chromosome elimination in the germ tissues leading to haploid spikes in 50% of the plants. The effect of the *Ph1* gene on meiosis is well known, and these results provide evidence for the role of *Ph1* in the maintenance of polyploid genome integrity. These and other data are discussed in relation to the structural and functional differentiation of S- and B-genome chromosomes and the practical utility of the stocks in wheat improvement.

One method to determine interchromosomal homologies, as pioneered by Sears [1954] in wheat, consists of measuring the gametophytic and sporophytic compensation of individual chromosomes for the corresponding missing chromosomes in nullisomic-tetrasomic (NT) combinations. If a substituted chromosome compensates for sporophytic (plant vigor and fertility) and gametophytic (as measured by gametic transmission) functions of the missing chromosome, then the chromosomes are classified as homoeologous. Conversely, if such plants lack vigor and are sterile, then the specific chromosomes are considered to be non-homoeologous. This allowed Sears [1966] to classify the 21 chromosomes of hexaploid wheat into 7 homoeologous groups, each containing one chromosome each from the A, B and D genomes.

This method of determining interchromosomal homologies has been extended to many species and genera

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of the Triticeae such as *Thinopyrum elongatum* (Host) D.R. Dewey [Dvorak, 1980] and *Aegilops longissima* Schweinf. & Muschl. [Friebe et al., 1993], among many others. Such wheat-alien chromosome substitution lines are not only of great theoretical interest for elucidating evolutionary relationships, but also of immense practical interest for capturing the rich allelic diversity of donor species for crop improvement [for review, see Friebe et al., 1996].

Aegilops speltoides Tausch (2n = 2x = 14, SS) is an outbreeding species belonging to the section *Sitopsis* and is native to the Mediterranean and the Middle East [van Slageren, 1994]. The S genome of *Ae. speltoides* is closely related to the B and G genomes of polyploid wheat [Sarkar and Stebbins, 1956; Friebe and Gill, 1996; Tsunewaki, 1996; Dvorak, 1998]. Previously, we reported the development of a complete set of *Triticum aestivum-Ae. speltoides* chromosome addition lines [Friebe et al., 2000]. Here, we report the production of a set of disomic S(B)-genome chromosome substitution lines to analyze the ability of the S-genome chromosomes to compensate for the loss of homoeologous B-genome chromosomes.

Materials and Methods

The disomic chromosome addition (DA) lines DA1S to DA7S containing a pair of each of the S-genome chromosomes of *Ae. speltoides* added to *T. aestivum* L. cv. Chinese Spring (CS) [Friebe et al., 2000] were crossed to the corresponding CS monosomic (M) stocks M1B to M7B. The chromosome constitutions of the F_1 plants were determined in root tip meristems, and plants double monosomic for an S-genome and a homoeologous B-genome chromosome with 2n = 42 chromosomes were self-pollinated. Thus, the F_1 plants of M1B × DA1S with 2n = 42 at metaphase I of meiosis will have a pairing configuration of 20 bivalents (20^{II}) + a 1S univalent ($1S^{I}$) + a 1B univalent ($1B^{I}$). The $20^{II} + 1S^{I} + 1B^{I}$-plants, assuming expected univalent behavior at anaphase I (they reach a pole 25% of the time and are lost as laggards 75% of the time), would produce 4 types of gametes, i.e. 1n = 20, 1n = 20 + 1B, 1n = 20 + 1S, and 1n = 20 + 1S + 1B in a ratio of 9:3:3:1. There is no competition among different types of gametes during female gametogenesis and all gametes are expected to function in the expected ratio. However, in male meiosis, because of strong competition for pollen tube growth and fertilization, euploid gametes with 20 + 1B or 20 + 1S (assuming 1S has all the genes that compensate for the missing 1B) function almost exclusively. Thus, 25% of all F_2 plants with 2n = 42 should have the chromosome complement of CS wheat (2n = 42) and another 25% should be DS1S(1B) having a pair of 1S chromosomes substituting for a pair of 1B chromosomes. Similar results are expected for all other chromosome combinations.

Progenies of the F_1 plants with 2n = 42 chromosomes were analyzed by C-banding and fluorescent in situ hybridization (FISH) to identify plants that were disomic chromosome substitution lines, in which a pair of S-genome chromosomes replaced a pair of homoeologous B-genome chromosomes. C-banding and chromosome identification was according to Gill et al. [1991], and FISH using the *Ae. speltoides*-derived clone pGc1R-1 was according to Friebe et al. [2000] and Zhang et al. [2002]. Meiotic metaphase I pairing was analyzed in pollen mother cells (PMCs) after staining with aceto-carmine. Sporophytic compensation in DS lines was determined as the number of seeds per spikelet in 3 spikes per plant, and 3–4 plants per line were analyzed.

Genomic DNA was extracted from the 1S to 7S wheat-*Ae. speltoides* disomic substitution lines and 21 CS nullisomic-tetrasomic (NT) stocks and digested with 4 different restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, and *Dra*I). RFLP probes used in the present study are listed in table 1. Southern hybridization was performed as described by Qi et al. [2003].

Table 1. List of RFLP probe and enzyme combinations used in the present study

Probe/enzyme combinations	
BCD1434-1S/*Hin*dIII	PSR945-5S/*Hin*dIII
PSR544-1L/*Eco*RV	PSR360-5L/*Eco*RI
BCD433-2S/*Dra*I	PSR580-5L/*Eco*RV
PSR388-2L/*Dra*I	PSR627-6S/*Dra*I
PSR909-3S/*Hin*dIII	BE443529-6S/*Eco*RI
BCD589-3L/*Hin*dIII	BE426401-6S/*Eco*RI
PSR584-4S/*Eco*RI	CDO497-6L/*Hin*dIII
PSR144-4S/*Hin*dIII	CDO595-7S/*Eco*RV
PSR920-4L/*Dra*I	PSR129-7L/*Dra*I
CDO1333-4L/*Hin*dIII	PSR311-7L/*Eco*RI

Results

The Structural Integrity of S-Genome Chromosomes in S(B) Substitution Lines

The structural integrity of the S-genome chromosomes in the S(B) substitution lines was assayed by C-banding, FISH analysis with the S-genome derived repeat pGc1R-1 [Friebe et al., 2000] and by molecular markers. The C-banding and pGc1R-1 FISH patterns of the *Ae. speltoides* chromosomes, except for 6S in the substitution lines, are identical to those present in the corresponding addition lines, indicating that they were not rearranged (fig. 1). However, chromosome morphology, C-banding and FISH patterns of chromosome 6S indicate that this chromosome has a terminal deletion in the short arm, which resulted in the loss of the satellite of 6S. A complete 6S chromosome spontaneously substituted for wheat chromosome 6A in DS6S(6A) as described previously [Friebe et al., 2000].

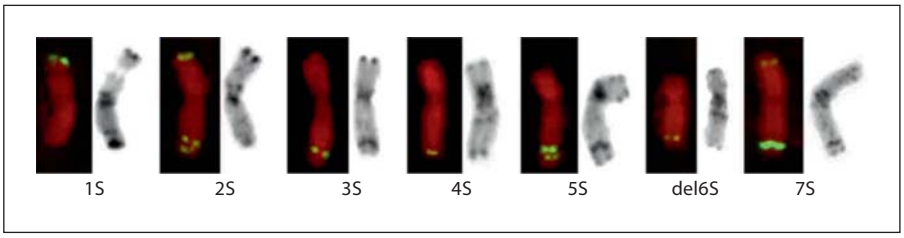

Fig. 1. pGc1R-1 FISH (left) and C-banding patterns (right) of the *Ae. speltoides* chromosomes present in the set of wheat-*Ae. speltoides* disomic substitution lines.

The identity of the *Ae. speltoides* chromosomes in the substitution lines was also verified by molecular markers from the 7 wheat homoeologous groups (table 1). The missing of a corresponding B-genome-specific fragment in each substitution line confirmed that the B-genome chromosome was replaced by an S-genome chromosome. Figure 2 shows an example of a Southern hybridization using the group-1 long arm marker PSR544 on a set of wheat-*Ae. speltoides* disomic substitution lines. The disomic DS1S(1B) substitution line is missing the 1B-specific band, but has an additional *Ae. speltoides* band, thus confirming the chromosomal constitution of this line. RFLP probe PSR679 is polymorphic for the 6S chromosome of *Ae. speltoides* and was previously mapped to the distal region of the group-6 short arm [Friebe et al., 2000; Weng et al., 2000]. This probe did not detect a 6S fragment in the selected disomic DS6S(6B) line; however, 2 EST probes, BE443529 and BE426401, that were previously mapped to the proximal regions of the group-6 short arm (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi), did detect the 6S fragments in DS6S(6B). The data confirmed that the disomic DS6S(6B) stock suffered from the loss of the distal segment of the short arm.

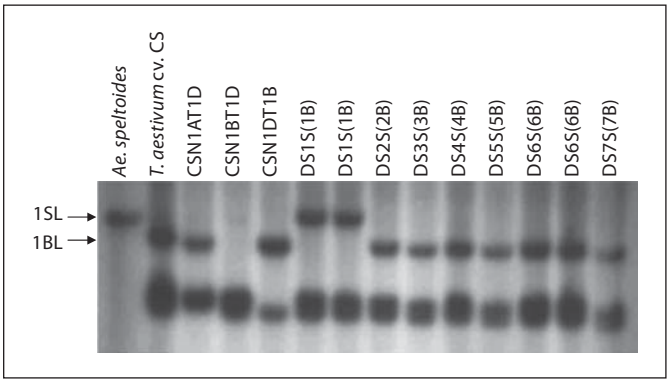

Fig. 2. Southern hybridization of the homoeologous group-1L probe PSR544 to *Hin*dIII-digested genomic DNA of *Ae. speltoides* accession 829, *T. aestivum* cv. Chinese Spring, the group-1 CS nullisomic/tetrasomic stocks, and the set of wheat-*Ae. speltoides* disomic substitution stocks. The top fragment was only present in *Ae. speltoides* and the disomic substitution DS1S(1B) stock, indicating it is 1S-specific. The second fragment was absent in N1BT1D and DS1S(1B), indicating that chromosome 1B is replaced by chromosome 1S in the DS1S(1B) stock.

Gametophytic Compensation

Data on the number of plants with 2n = 42 chromosomes and S-genome chromosome substitutions recovered in the progeny of S- and B-genome double monosomics for the 7 chromosomes are summarized in table 2. The recovery of 42-chromosome plants was as expected except for the 3B/3S and 5B/5S combinations, which had significantly fewer plants with 2n = 42 chromosomes. As mentioned previously, one quarter of the 2n = 42 chromosome plants should be disomic S-genome substitutions for the homoeologous B-genome chromosomes. Although S-genome disomic substitutions were recovered for all chromosomes tested, the frequency of recovery was significantly lower than expected for all chromosome combinations and was statistically significant in the combinations of 1S(1B), 3S(3B), 5S(5B) and 6S(6B) (table 2). The data indicate either significant disturbances in meiotic behavior that led to the low recovery of 42-chromosome plants in certain combinations or poor gametophytic compensation for all S-genome chromosomes during the phase of pollen growth and fertilization.

Sporophytic Compensation

Two aspects of sporophytic compensation affecting meiotic pairing and spike fertility were measured for all the seven S(B) chromosome substitution lines (table 3). Metaphase I pairing of the disomic substitution lines is shown in figure 3 and summarized in table 3. All disomic substitution lines except DS4S(4B) and DS5S(5B) showed normal metaphase I associations and usually paired as bivalents with only a few cells having univalents. The number of cells with univalents was higher in DS4S(4B), however, it is unknown if the higher frequency of univalents in this line is contributed by 4S or by wheat chro-

Table 2. Chromosome constitutions of F_2 progeny from crossing Chinese Spring B-genome monosomic stocks with the homoeologous wheat-*Ae. speltoides* disomic addition stocks

Cross	No. of F_2 plants screened	Plants with 2n = 42			Disomic substitution		
		Expected	Observed	χ^2	Expected	Observed	χ^2
M1B × DA1S	60	23	21	0.174	5.75	1	3.924*
M2B × DA2S	45	17	13	0.941	4.25	1	2.485
M3B × DA3S	75	29	17	8.470**	7.25	1	5.388*
M4B × DA4S	30	11	9	0.364	2.75	1	1.114
M5B × DA5S	100	38	19	9.500**	9.5	2	5.921*
M6B × DA6S	120	45	38	1.089	11.25	1	9.339**
M7B × DA7S	45	17	12	1.471	4.25	1	2.485

* and ** indicate significant differences between the observed and the expected number at the 5% and 1% levels, respectively.

Table 3. Meiotic metaphase I associations observed in PMCs and spikelet fertility of the set of disomic wheat-*Ae. speltoides* substitution lines

Line	No. of plants	PMCs with 21^{II}	PMCs with univalents	PMC with multivalents	Seeds per spikelet
CS	3	54	1 (1.8%)		2.76
DS1S(1B), TA6651	3	65	10 (13%)		2.72
DS2S(2B), TA6652	3	91	6 (6.2%)		1.48
DS3S(3B), TA6653	3	112	4 (3.4%)		2.87
DS4S(4B), TA6654	3	135	67 (33.2%)		3.01
DS5S(5B), TA6655, diploid	2	1	10 (41.7%)	13 (54.2%)	0.70
DS5S(5B), haploid	2		14 (53.8%)	12 (46.2%)	sterile
DS6S(6B), TA6656	3	113	12 (9.6%)		2.83
DS7S(7B), TA6657	3	68	4 (5.6%)		2.38

mosomes. DS1S(1B) also showed univalents in 13% of the PMCs.

Two types of plants were observed in DS5S(5B). Chromosome counts in root tip meristems showed that 3 plants had 2n = 42 chromosomes, whereas 1 plant had 2n = 41 + t (41 chromosomes plus 1 telosome). At meiotic metaphase I, one of the 2n = 42 chromosome plants and the plant with 2n = 41 + t showed the expected chromosome constitution, whereas the remaining 2n = 42 chromosome plants had a haploid chromosome constitution (fig. 3e, f). Both types of plants had univalents and multivalents. The diploid plants were partially fertile, but the haploid plants were completely sterile.

Spike morphologies of the wheat-*Ae. speltoides* substitution lines are shown in figure 4. Spike morphology of DS1S(1B), DS3S(3B) and DS4S(4B) were similar to that of CS. The upper part of the spike of DS2S(2B) was poorly developed. The spike of DS6S(6B) was awned. The spike of DS7S(7B) was more lax compared to CS.

The sporophytic compensation abilities of the *Ae. speltoides* chromosomes for B-genome chromosomes of wheat were determined in 3 plants with 3 spikes per plant as the number of seeds per spikelet (table 3). The number of seeds per spikelet in DS1S(1B), DS3S(3B), DS4S(4B), DS6S(6B), and DS7S(7B) was similar to that of CS, indicating that the *Ae. speltoides* chromosomes in these stocks compensated well for the loss of their homoeologous B-genome chromosomes. Fewer seeds per spikelet were observed in DS2S(2B) and DS5S(5B). The reason for poor sporophytic compensation ability of chromosome 2S for 2B of wheat is unknown, but the poor compensation ability of 5S for 5B of wheat is caused by disturbed meiosis.

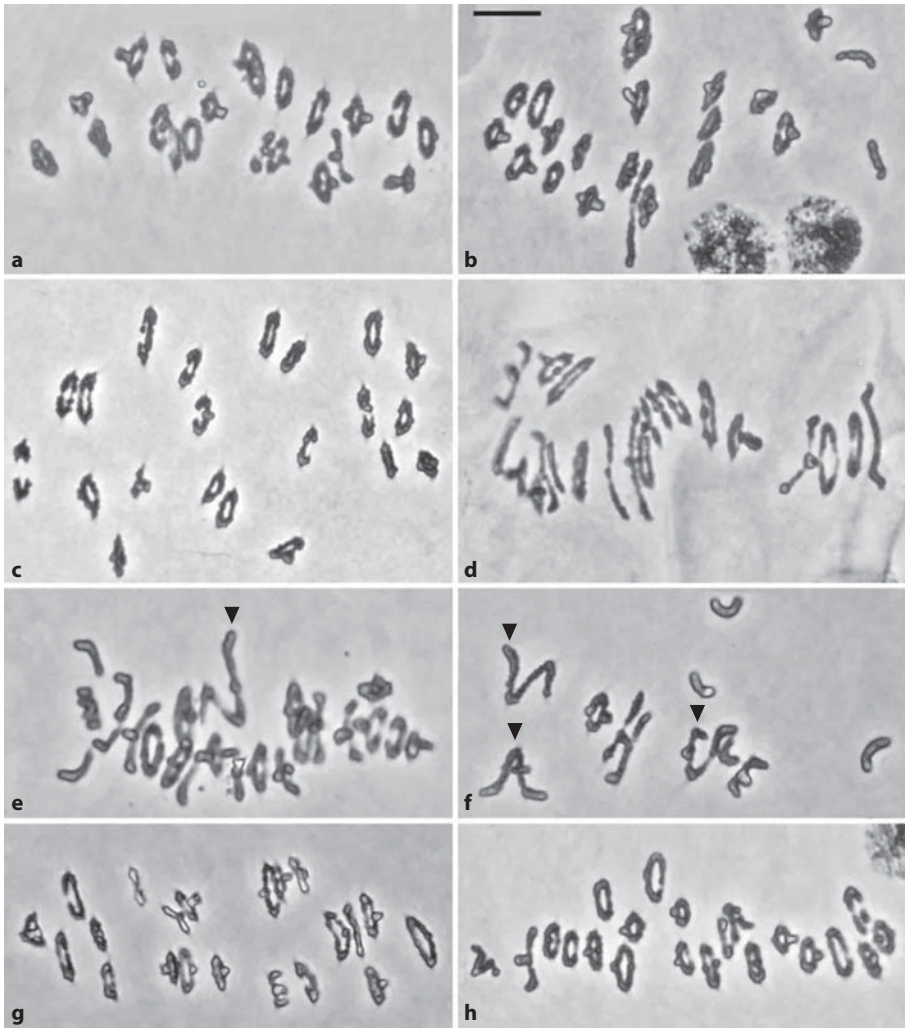

Fig. 3. Meiotic metaphase I pairing behavior of disomic substitution (DS) lines, where S-genome chromosomes of *Ae. speltoides* substitute for the B-genome chromosomes of wheat. **a** DS1S(1B), 21^{II}; **b** DS2S(2B), $20^{II} + 2^{I}$; **c** DS3S(3B), 21^{II}; **d** DS4S(4B), 21^{II}; **e** DS5S(5B), $19^{II} + 1^{IV}$; **f** DS5S(5B), $4^{II} + 3^{III} + 4^{I}$; **g** DS6S(6B), 21^{II}; and **h** DS7S(7B), 21^{II}. Scale bar represents 10 μm, multivalents are marked by arrowheads.

Fig. 4. Spike morphologies of *T. aestivum* cv. CS-*Ae. speltoides* disomic substitution lines.

Discussion

Ae. speltoides has been considered the most closely related donor species for the G/B genomes of polyploid wheats [Kimber, 1981; Tsunewaki and Ogihara, 1983; Gill and Chen, 1987; Ogihara and Tsunewaki, 1988; Badaeva et al., 1990, 1996a, b; Tsunewaki et al., 1990; Jiang and Gill, 1994a, b; Friebe and Gill, 1996; Dvorak, 1998]. These studies also revealed greater homology of the S genome of *Ae. speltoides* with the G genome of *T. timopheevii* (Zhuk.) Zhuk. than with the B genome of *T. turgidum* L. and *T. aestivum*. However, the B and G genomes of polyploid wheats are more closely related to each other than to any of the diploid progenitor genomes. Our overall results on gametophytic and sporophytic compensation of S-genome chromosomes for the B-genome chro-

mosomes support this view of genomic relationships. For example, *T. timopheevii* is the only other wheat species that harbors the *Ph1* gene because its 5G chromosome completely compensates for 5B in DS5G(5B) plants that have diploid-like meiosis [Dhaliwal, 1977]. In contrast, the DS5S(5B) plants had a disturbed meiosis indicating *Ae. speltoides* lacks the *Ph1* gene or it is polymorphic. Alternately, the *Ph1* gene arose following polyploidization.

A similar lack of compensation for gametophytic and sporophytic gene functions can be deduced for other S-genome chromosome substitutions for homoeologous B-genome chromosomes. Chromosome 1S in DS1S(1B) showed poor gametic transmission (table 2) and its sporophyte had 13% univalents at meiotic metaphase I. Chromosome 2S substituting for 2B failed to produce a normal spike. Chromosome 3S had poor gametophytic compensation and chromosome 4S had a high rate of univalents during meiosis. Chromosome 5S, besides lacking the *Ph1* gene function, also did not compensate for gametic functions that led to poor transmission of gametes carrying chromosome 5S. The 6S(6B) substitution, besides poor sporophytic and gametophytic compensation, also showed chromosome instability. The 6B chromosome in CS wheat carries an awn suppressor gene that is missing in 6S and leads to an awned phenotype in DS6S(6B) plants. The 7S chromosome had a lax spike. These observations support the structural and functional differentiation between B- and S-genome chromosomes.

In some cases, specific genes and allelic variation and, in other cases, gene interactions and epigenetic effects may be responsible for the observed phenotypes. Gametocidal factors *(Gc)* in wheat are strong segregation distorters [Endo and Tsunewaki, 1975; Maan, 1975; Endo, 1990]. Three *Gc* genes were reported in *Ae. speltoides*. Two *(Gc1a* and *Gc1b)* are located on 2S and another one is on 6S [Tsujimoto and Tsunewaki, 1984, 1988; Kota and Dvorak, 1988]. Plants hetero- or hemizygous for a *Gc* factor produce 2 types of gametes, with or without the *Gc* factor. The *Gc* genes on 2S and 6S have different modes of action. The *Gc* gene on chromosome 2S kills the gamete lacking it and, therefore, is exclusively transmitted to the offspring, whereas the *Gc* gene on chromosome 6S causes chromosome breakage in gametes carrying the *Gc* gene. There is no evidence for the presence of a *Gc* gene on chromosome 2S in our material and, thus, the reduced fertility in DS2S(2B) is not caused by *Gc* gene action. Instead, the reduced fertility is caused by an unknown genetic factor that leads to abnormal spike development. However, it is possible that chromosome 6S may carry a *Gc* gene because we identified one DS6S(6B) plant in 38 F_2 plants with 42 chromosomes. The recovered chromosome 6S in the DS6S(6B) stock was homozygous for a small deletion in the distal part of the 6S short arm, whereas 6S in the DS6S(6A) stock was identical to that present in the disomic 6S chromosome addition line.

Dong et al. [2002] reported that a wheat DNA mismatch repair gene, *TaMSH7*, a homologue of the *MSH6* gene in mammals and yeast, is located on the short arms of group-3 chromosomes. The DNA mismatch repair system plays a critical role in maintaining genetic stability in bacteria and higher eukaryotes. The poor gametophytic compensation of 3S for 3B may be caused by the *MSH* gene. We do not know if *TaMSH7* is present in 3S, but the meiotic pairing in DS3S(3B) is normal, ruling out the possibility that the missing *TaMSH7* in 3BS causes poor transmission of gametes with 3S.

Studies on meiotic pairing in PMCs and fertility of a set of S(B) disomic substitution lines indicated that most S-genome chromosomes compensated well for the loss of their homoeologous B-genome chromosomes in a wheat background, except for DS5S(5B) in which the poor compensation ability of 5S for 5B of wheat is caused by disturbed meiosis. The number of PMCs with univalents ranged from 3.4% (DS3S) to 33.2% (DS4S) in 6 disomic substitution lines compared to 1.8% in CS wheat. The number of seeds per spikelet ranged from 1.48 (DS2S) to 3.01 (DS4S) compared to 2.76 in CS. Interestingly, despite some meiotic disturbance observed in DS4S(4B), this line was highly fertile indicating the existence of fertility genes per se.

In the present study, we observed chromosome elimination in some DS5S(5B) plants. Two plants of DS5S(5B) with 42 chromosomes in root tip meristems had a haploid meiotic metaphase I chromosome constitution (21 chromosomes, fig. 3f). There appears to be a genetic trigger in the germ cells that leads to elimination of one set of 21 chromosomes producing haploid plants. Because these plants are missing chromosome 5B and the *Ph1* gene, it is tempting to speculate that *Ph1* plays a role in maintaining genomic integrity in polyploid plants. Further studies are needed to study the mechanism of chromosome elimination.

In conclusion, the isolation of a complete set of *Ae. speltoides* substitution lines in hexaploid wheat background now opens the possibility of studying the structural and functional differentiation of the S-genome chromosomes by genomics and epigenomic approaches. The DS5S(5B) line particularly will be valuable for analyzing the mechanism of *Ph1* action. These materials are

also useful for wheat improvement. Screening of these lines has already identified resistance to stem rust race Ug99 [Xu et al., 2008]. Seed samples of the wheat-*Ae. speltoides* substitution lines are available from the Wheat Genetic and Genomic Resources Center at Kansas State University.

Acknowledgements

We thank John Raupp for editorial assistance and Duane Wilson for excellent technical help. This research was supported by grants from the Kansas Wheat Commission, the Kansas Crop Improvement Association, a special USDA-CSREES grant to the Wheat Genetic and Genomic Resources Center. This paper is contribution number 11-195-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506-5502.

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